



## Acute Toxicity Studies of various Leaves Extracts of *Cupressus torulosa* D. Don ex Lamb. and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li

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### ABSTRACT

The present work aims to investigate the acute toxicity profile selected medicinal plants. In the present study petroleum ether, chloroform, ethanolic and aqueous extract of *Cupressus torulosa* D. Don ex Lamb. (Leaves) and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li (Leaves) were evaluated for acute toxicity studies using OECD guidelines 423. The results indicate that all the extract at the dose of 2000 mg/kg b.w. are considered as safe.

**Keywords:** *Cupressus* species, Acute Toxicity Studies, Dose

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### INTRODUCTION

Ayurveda deals with the study of India medicinal plants and usually it is considered as herbal drugs are less toxic or don't have any toxicity [1,2]. But the better use of these drugs there scientific validation need to be established. The present study was designed to evaluate the two Indian medicinal plants widely used to treat the various disorders as mentioned in traditional system of medicine.

*Cupressus torulosa* D. Don ex Lamb. (Himalayan cypress or Bhutan cypress), is native to the mountainous northern regions of the Indian Subcontinent, primarily the Himalayas. It is a large tree, growing up to 45 m (150 ft) in height [3-4]. *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li. (Vietnamese golden cypress), is native to the Vietnam, also found in Himalaya regions. The tree is 10-15 m tall in height [5-8].

So, far no any systematic study was carried out to determine the acute toxicity of these plants, therefore, the present work was undertaken to reveal the effective dose.

### MATERIAL AND METHODS

#### Collection of herbs and their authentication

The leaves of *Cupressus torulosa* D. Don ex Lamb. (CTL) and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li. (CVL) were collected in the months of July-December 2020 from the Himalaya region and identified & authenticated by Dr. S. N. Dwivedi, Retd. Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, (M.P.) and was deposited in our Laboratory. Voucher specimen No. J/Bot/2020-CTL012 & CVL013 was allotted.

#### Successive Extraction of selected herbs

Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered plant material (250gms) were loaded in Soxhlet apparatus and was extracted with petroleum ether (60-62°C), Chloroform, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined [9].

#### Acute Toxicity Studies of Extracts

Organization for Economic co-operation and Development (OECD) regulates guideline for oral acute toxicity study. It is an international organization which works with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing [10].

Following are the main type of guideline followed by OECD

- Guideline 420, Fixed dose procedure. ( 5 animals used )
- Guideline 423, Acute toxic class. ( 3 animals used )
- Guideline 425, Up and down method. (1 animal used)

#### Procurement of experimental animals

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee, after scrutinization.

#### RESULTS AND DISCUSSION

The petroleum ether, chloroform, ethanolic and aqueous extract of *Cupressus torulosa* D. Don ex Lamb. (Leaves) and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li (Leaves) were screened for acute toxicity studies as per OECD guidelines 423 for the determination of LD<sub>50</sub> and ED<sub>50</sub>. The results (Table 1 & 2) indicates that all extract showed no any toxicity at the dose of 2000 mg/kg body weight, hence the LD<sub>50</sub> for the selected drugs was 2000 mg and ED<sub>50</sub> was 1/10<sup>th</sup> of LD 50 i.e., 200 mg. As per OECD423 guidelines the dose is said to be “unclassified” under the toxicity scale. Hence further study with higher doses was not executed.

The acute toxicity study showed that petroleum ether, chloroform, ethanolic and aqueous extract of CTL and CVL produced no toxic effects as evidenced by the absence of signs of toxicity (or) mortality in the animals during the study period (14 days of observation). Additionally, no weight losses, alternation of consumption of pellet or macroscopic alterations in the viscera of treated animals were detected. The similar results were depicted by Chavan *et al.*, [11] while evaluation toxicity studies of some Indian medicinal plants and Patel *et al.*, [12]. The result for acute oral toxicity study for is presented in Table. 2

**Table 1: Determination of LD<sub>50</sub> and ED<sub>50</sub> of Extract of *Cupressus torulosa* D. Don ex Lamb. (Leaves)**

S/No.	No. of Animals	Extract Dose (mg/kg)	No. of death of animals			
			PEECTL	CECTL	EECTL	AECTL
1.	3	5	0	0	0	0
2.	3	50	0	0	0	0
3.	3	300	0	0	0	0
4.	3	2000	0	0	0	0
5.	3	5000	0	0	0	0

**Table 2: Determination of LD<sub>50</sub> and ED<sub>50</sub> of Extract of *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li (Leaves)**

S/No.	No. of Animals	Extract Dose (mg/kg)	No. of death of animals			
			PEECVL	CECVL	EECVL	AECVL
1.	3	5	0	0	0	0
2.	3	50	0	0	0	0
3.	3	300	0	0	0	0
4.	3	2000	0	0	0	0
5.	3	5000	0	0	0	0

**Table 3: Acute toxicity study of *Cupressus torulosa* D. Don ex Lamb. (Leaves) and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li (Leaves)**

Parameters	Duration of observations							
	1 hr	2 hr	3 hr	4 hr	8 hr	24 hr	8 <sup>th</sup> Day	14 <sup>th</sup> Day
Appearance	N	N	N	N	N	N	N	N
Activity	P	P	P	P	P	P	P	P
Touch	++	++	++	++	++	++	++	++
Sound	++	++	++	++	++	++	++	++
Light	++	++	++	++	++	++	++	++
Lacrimation	A	A	A	A	A	A	A	A
Salivation	A	A	A	A	A	A	A	A
Licking of paw	A	A	A	A	A	A	A	A

Abbr.: N = Normal, A = Absent, P = Present, + = minimum, ++ = present medium

## CONCLUSION

From the results obtained it was concluded that the selected plants i.e., *Cupressus torulosa* D. Don ex Lamb. (Leaves) and *Cupressus vietnamensis* (Farjon & Hiep) Q.P. Xiang & J. Li (Leaves) are free from the toxicity and are considered as safe at the dose of 2000 mg/kg body weight. Thus, these plants may be used for the formulation at the dose mentioned.

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